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SITE OF ACTION OF BACTERIAL PYROGEN IMMUNOFLOUORESCENT STUDIES IN MICE

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Received for publication May 28, 1964

Studies on the distribution of bacterial endotoxin in animal tissues have been developed by Braude *et al.* (1, 2) as early as in 1955 who found ^{51}Cr -labelled endotoxin from *E. coli* taken up by the buffy coat layer as well as by the RES cells. Rowley and Jenken (3) and Noyes *et al.* (4) have also undertaken a similar procedure, but have obtained a little different results from each other. When such a high-molecular agent as the bacterial endotoxin will be labelled with radioisotope, however, a question will arise if there might occur unexpected biotransformation or molecular configuration in the endotoxin structure. On the other hand, Cremer and Watson (5) have demonstrated the endotoxin of *S. typhosa* present in the RES cells in mice by means of Coons' fluorescent antibody technique. Our present aim is to determine the exact site of action of bacterial endotoxin in more purified form by the Coons' method.

Lipopolysaccharide from *Ps. fluorescens* (T.T.G., Fujisawa Pharmaceutical Co., Ltd.) was employed as a bacterial pyrogen. The rabbit was immunized with T.T.G. which had been previously purified either by the alum precipitation or by the Freund's incomplete adjuvant. After the immune specificity of antisera against T.T.G. was confirmed by gel-diffusion test (Fig. 1A), the anti-T.T.G. fluorescent conjugate was prepared by labelling the crude globulin of the antisera with fluorescein isothiocyanate dye. Male albino mice weighing 20 to 23 g were injected i.v. with 100 to 300 $\mu\text{g}/10\text{g}$ of T.T.G. and blood smear preparations and frozen sections of various tissues (3 to 5 μ thick) were stained by the anti-T.T.G. fluorescent conjugate.

Result : 1) With the blood smear the bacterial pyrogen was shown to exist only in the cytoplasm of polymorphonuclear leucocytes (Fig. 1B), not in those of erythrocytes, monocytes and lymphocytes, while the one which was stained with anti-T.T.G. fluorescent conjugate adsorbed with T.T.G. antigen was negative (Fig. 1C). The blood smear from intact mice which was similarly stained showed also negative results. From these findings it was determined that the particles thus taken up and stained in leucocytes were the bacterial pyrogen *per se*. The uptake of bacterial pyrogen was occurred initially in the polymorphonuclear leucocytes 10 minutes after the administration of T.T.G. and the destruction of cell wall and the efflux of bacterial pyrogen were observed about 1 hour after T.T.G.

2) With the frozen sections the bacterial pyrogen was also found in Kupffer cells of the liver, reticulum cells of the spleen, glomeruli and tubular epithelium of the kidney, interstitium of the lungs, intravascular space, wall of the vessels, and leucocytes as well as macrophages in various tissues, while none was shown in the heart muscle and parenchymal cells in brain.

FIG. 1. A) Immunospecificity between antisera and T.T.G. antigen in gel-diffusion test. See clear band shown between antisera (center) and T.T.G. antigen.

B) The uptake of bacterial endotoxin in the leucocytes 30 min after T.T.G. injection. Bright fluorescence of T.T.G. antigen is localized in the neutrophile leucocytes immunospecifically.

C) A leucocyte stained with anti-T.T.G. conjugate adsorbed with T.T.G. antigen.

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